Impact of the combination of larval control and Indoor Residual Spraying on *Anopheles gambiae* density and vector capacity for human malaria. (Mali)

Integrated Vector Management (IVM) Task Order 2

Contract GHA-I-02-04-00007-00

Prepared for:
United States Agency for International Development

Prepared by:
RTI International
3040 Cornwallis Road
Post Office Box 12194
Research Triangle Park, NC 27709-2194

June 2010

University of Bamako, Mali

Faculty of Medicine, Pharmacy and Odonto-Stomatology,
BP: 1805, Tel: (+223) 7536 9580, Fax: (+223) 2022 4789,

Malaria Research and Training Center (MRTC)-Entomology
Vector Genomics and Proteomics laboratory

Project Title: **Integrated vector management: Impact of the combination of larval control and Indoor Residual Spraying on *Anopheles gambiae* density and vector capacity for human malaria.**

June 2010

Final Deliverable

Contact: Mamadou B. Coulibaly, Ph.D., Pharm.D
E-mail: doudou@icermali.org
Coulibaly7@gmail.com

US President's Malaria Initiative

Table of Contents

Abbreviations and Acronyms

CSCOM: Centre de Santé Communautaire (Community Health Center)
CDC: Center for Disease Control
GPS: Geographical Positioning System
GIS: Geograpgical Information System
IRB: Institution Review Board
IRS: Indoors Residual Spraying
ITNs : Insecticide-Treated Nets
Km: Kilometer
MRTC: Malaria Research and Training Center
Nb: Number
NMCP/DPNLP: National Malaria Control Program (Direction du Programme National de Lutte Contre le Paludisme)
PDA: Personnal Digital Assistant
PI: Principal Investigator
PMI : President's Malaria Initiative
PSC: Pyrethrinum Spray Catch
RTI: Research Triangle Institute
USA: United States of America
USAID: United States Agency for International Development
LMVR/NIAID/NIH: Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health
MSC: Mali Services Center
% : Percentage

1. Introduction

Malaria prevention has relied principally on controlling the adult populations. Indoor residual spraying may reduce by as much as 90 % entomological measures of transmission with a concomitant reduction of malaria incidence but is not sufficient to eliminate transmission. Complementary tools to IRS and ITNs are needed to target other stages and sources of mosquitoes to synergize with IRS and ITNs in lowering transmission levels but also should be developed as part of an insecticide resistance management program. Larval control has largely been dismissed as a potential tool because of the wide range of potential larval habitats and the transient nature of such habitats. Nevertheless, recent studies in Kenya and other countries in sub-Saharan Africa have shown that the vast majority of larval habitats that produce adults are man-made habitats located near human dwellings and capable of holding water for several weeks, although the naturally occurring larval sites formed from receding water of the river is an exception to this. This implies that bodies of water that are likely to produce adults can be targeted effectively.

The Mali National Malaria Control Program (NMCP/DPNLP) with the support of the US Agency for International Development (USAID) under the PMI, in collaboration with the Center for Disease Control (CDC-Atlanta), has agreed to initiate a study that **aims at assessing the added value of larviciding to IRS**. The main objectives could be categorized into three parts: 1) the training of the trainers and the training of the spray operators; 2) the actual spray operations and 3) the information and sensitization of the population of the region of Koulikoro about larviciding.

This was a pilot study conducted in the Koulikoro region by the entomologic department of MRTC (Malaria Research and Training Center). The end goal is to advise the National Malaria Control Programme on integrating larval control into the national malaria control strategy based on scientific evidence.

2. Objectives

a. Train the trainers and spray operators and map mosquito breeding sites

b. Compare entomological parameters between areas with larviciding+IRS and areas with IRS only

- Monitor selected breeding sites to determine the larval frequencies (presence/absence) and abundance
- Determine adult mosquito densities in human dwellings
- Determine the entomological inoculation rates in both settings (areas with larviciding+IRS and areas with IRS only)

c. Inform and sensitize leaders and communities on the importance of larviciding

3. Methods

3.1. Study sites and their selection justification

Study sites

This study is being carried out in the health district of Koulikoro located at 60 km east of Bamako where two rounds of district-wide IRS have been conducted (in July-August 2008 and June-July 2009). The district is located in North Sudan Savanna areas where, malaria transmission is seasonal (June to October, corresponding to the rainy season) and peaks at the end of the rainy season. The prevalence of malaria infection in children under 5 years old varies from 30-40% during the dry season (December to May) and may reach 60 to 70% during the rainy season. The Entomological inoculation rate is below 1 infective bite per person per month during the dry season when infected anopheline mosquitoes are barely detectable; and reach 30-40 infected bites per person per month during the rainy season. The health district is divided into 20 health zones (aire de santé). Each “aire de santé” encompasses 10 to 15 villages mostly located within a radian of 15 to 20 km from a community health center (CSCOM). Each “aire de santé” regroups a population size of 10 to 20,000 inhabitants in average. Figure 1 show the Koulikoro region inside Mali and the relative positions of the six villages.

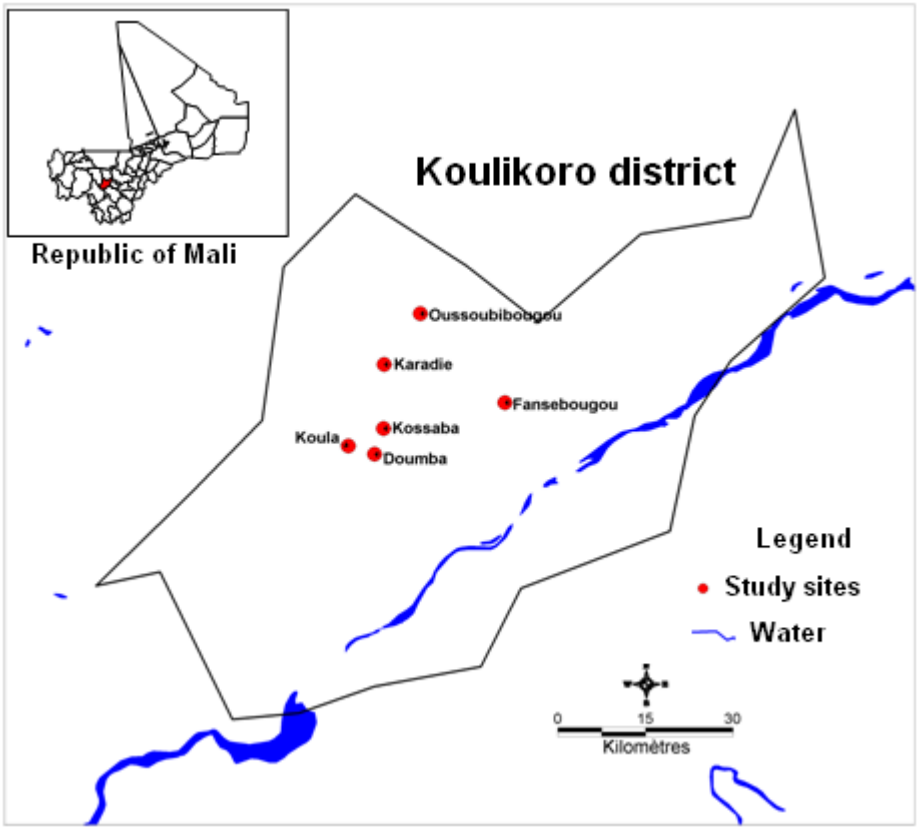


Figure 1: Study villages: Koula, Doumba and Kossaba in the Koula health zone, and Oussoubibougou, Fansebougou and Karadie in the Sirakorola health zone

Selection justification

Two contiguous health zones or "aire de santé" (Koula and Sirakorola) were selected for the study based on geographical accessibility (all seasons) and size. The rational for this choice is that villages of the same "aire de santé" tend to be comparable with regard to ecological characteristics and transmission pattern. In each "aire de santé" three villages were selected and randomly assigned to IRS or to IRS+Larviciding (see figure below). Villages are, at least, 3 km apart from each other.

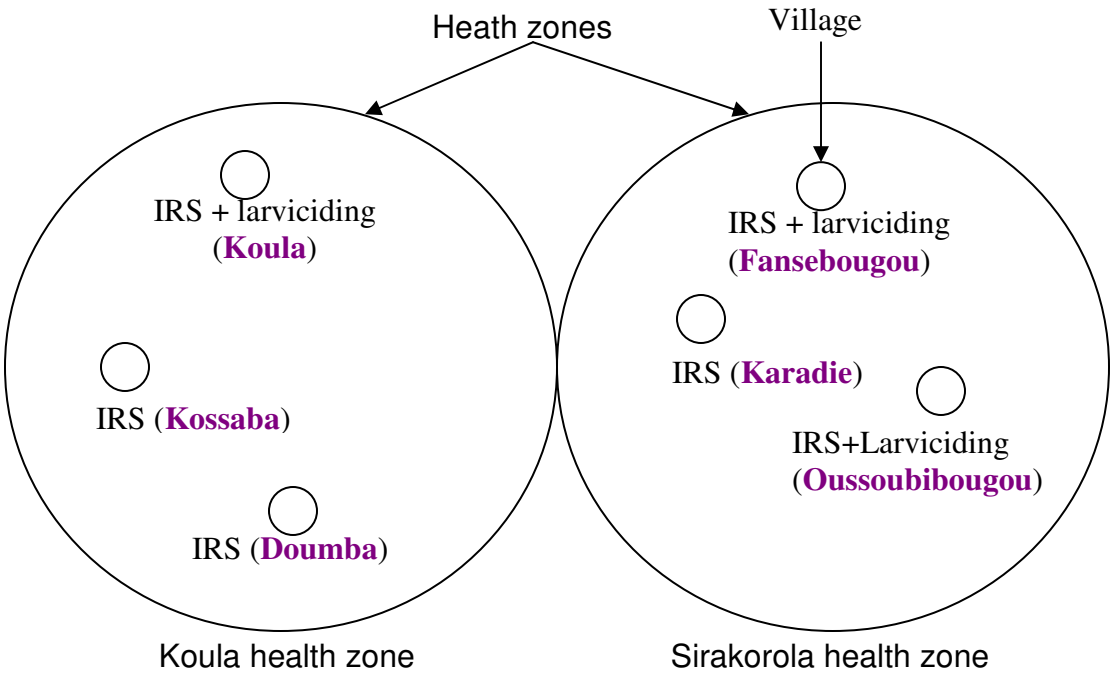


Fig 2: Randomly assigned villages to IRS or to IRS+Larviciding. Names in purple are the villages.

Study period

The actual larviciding started on June 20th 2009 and continues up to November 2009 according to the initial plan. IRS was conducted for the second time from May 16th to June 26th (RTI Bamako). These dates might change from locality to locality. Larviciding is being conducted by MRTC while Research Triangle International, Inc. (RTI), conducted IRS activities. RTI has experiences in conducting IRS activities in Angola, Senegal, Uganda and other countries and has been identified as USAID's principal implementing partner to provide support for the IRS activities in Mali.

3.2. Training

The training was done in two steps: the training of the trainers (TOT) and the training of the spray operators. Interactive lectures on malaria and the control strategies and practices were adopted as methods. The TOT was achieved on June 18th 2008. The trainer was Peter Dechant from Valent BioSciences (Illinois, USA). The training of the spray operator took place on June 4th 2009. The same methods were adopted. This was conducted by MRTC staff members (Dr. Coulibaly Mamadou, Dr. Diallo Brehima, Dr. Guindo Amadou, Dr. Traore Mohamed and Amadou S Traore) and the head of the health center of Sirakorola one the health zones to receive larvaiciding (Dr. Samake Michel).

3.3. Identification, characterization and mapping of breeding sites

Mapping was conducted from June 4th to June 16th 2009. A team of entomologists including a GIS technician prospected the villages of Oussoubibougou, Fansebougou, Koula (tests), Kossaba, Doumba and Karadie (control) for potential breeding sites. In each village entomologists and village guides identified brick pits, swamps, streams, tire traces as potential breeding sites. Geographical coordinates (longitude and latitude) for each site were recorded using GeoExplorer 3 (Trimble ™). This device has a three meter precision. In addition to the coordinates of the breeding sites, coordinates of reference points in the villages were also recorded. These include mosques, pumps schools, markets and so forth. The data were transferred to a computer (Dell) using GPS Pathfinder 2.9 software. Futures were visualized and then exported *ArcGis* 9 for mapping. PDAs were used to record characteristics of the breeding sites. These include identification number, size, location (in the sun, in the shade), presence/absence of water. Regarding the prospection for identifying potential breeding sites both inside and outside of the villages were explored. Outside refers to 300 m around the village from the very last houses counting from the center.

3.4. Monitoring and evaluation

Mosquito larvae

Aquatic stages including larvae and pupae were monitored in sentinel breeding sites before applying the bio-pesticide. This monitoring consisted in checking for the presence or absence of larvae and pupae in each sentinel site. Entomologists or trained local guides approached the breeding sites gently to not chase larvae or pupae (if they are present) away. Regardless whether or not larvae and or pupae were seen, entomologists proceeded with dipping using 300 ml dippers. Since we did not have the white bottom dipper the content of the dipper was transferred into a white pan to look for larvae and pupae. The presence/absence of larvae was recorded by instar (different developmental stages of larvae, there are four of them referred to as L1, L2, L3 and L4). This step is referred to as monitoring. The larval densities were determined based on 10 dipping in average. Some breeding sites were not big enough to allow ten dipping. Forty eight hours after the bio-pesticide application in the breeding sites entomologists visit the breeding sites again for evaluation. The same methods are conducted as mentioned above for determining the densities of the larvae and the pupae and their presence/absence. This step is referred to as evaluation.

Monitoring and evaluation were conducted in each of the three villages where Larviciding was combined with IRS. These villages will be referred to as test villages (they are Fansebougou, Oussoubibougou and Koula). In the three other villages only monitoring was conducted since there was no larviciding. These villages will be referred to as control villages (they are Kossaba, Karadie and Doumba). It is noteworthy that villages were randomly selected to be tests or controls.
Even though sentinel sites were selected for the monitoring and evaluation all the identified potential breeding sites were treated in the test villages.
Monitoring, treatment and evaluation were conducted every week. For the treatment details, see the "Larviciding section" below.
In addition to larval monitoring the number of new breeding sites was also recorded as well as their geographical coordinates.

Mosquito adults

Pyrethrinum spray catches using Premium® (Dichlorvos 1.20 %, fenitrothion 0.40% and tetramethrin 0.15%) were monthly conducted in twenty four (24) randomly selected houses in each of the six villages. The very same houses were visited in subsequent months to determine the impact of the combined larviciding-IRS on mosquito adult densities. The number of collected mosquitoes was recorded on a datasheet according to the abdomen stages (unfed, blood fed, half-gravid and gravid). This allows the estimation of the man biting rates (MBR) and the densities. Brought to the laboratory in Bamako mosquitoes were treated in ELISA to determine the *Plasmodium falciparum* infection status. A sample was also treated by PCR to determine the vector species and molecular forms.

3.5. Larviciding

Larviciding refers to the actual treatment of the breeding sites with bio-pesticides. *Bacillus thuringiensis israelensis* or Bti, under the brand name of Vectobac® was applied weekly to the breeding sites at a dosage of 400g/ha (125-500g/ha recommended by the manufacturer) after a mosquito larval survey. VectoBac® is brownish powder that containing Gram-positive soil bacterial toxin-producing spores that kill mosquito larvae by septicemia up on ingestion.
The dilution of the product was based on the dosage and other parameters such as the mean walking speed of the operator (meter/min), the swath (5m), the flow rate of the sprayer (ml/min; function of the type of nozzle), the coverage rate (ha/min) and the spray volume. The determination of these parameters is conducted through calibration, a step necessary for each sprayer. In our VectoBac® application we used the Star 16 Agro OSATU sprayer from GOIZPER s.coop.. They have a capacity of 16 liters. Standard nozzles were used and from the calibration exercise we came up with the figure of 86g or two scoops and half to dilute into 10 liters of water.
In each of the test villages an entomologist and 4-10 operators (depending on the rain frequency) visited the breeding sites. Treatment was conducted immediately after the larval and pupae monitoring. The evaluation was conducted 48 hours later.

3.6. Information and sensitization of the leaders and the population about the importance of larviciding in the Koulikoro region

Two meetings were scheduled in the Koulikoro region. The first one was done on May 17th 2010 in the Koulikoro district and was intended for politico-administrative leaders, religious leaders, leaders of the women's associations, representatives of the ministry of health, the ministry of environment, and the ministry of the social development. The meeting consisted of presentation of the national malaria control strategies by a representative of the NMCP (Dr. Sidibe Halidou), and presentation of larval control by Dr. Coulibaly Mamadou from MRTC. The latter presentation included the results of this pilot study.

The goal of this meeting was to inform the leaders so that they relay the information to their constituents. We expect from these leaders the development, based on the political will, of strategies for larval control in collaborations with the partners and the communities.

The second meeting was held on June 03rd and was intended for the community leaders in the villages where the actual larviciding study has been conducted. The expected result was for the leaders to understand the role of the communities in larval source management and relay the information to the populations.

3.7. Ethical considerations

The project was submitted to the institution review board (IRB) of the Faculty of Medicine, Pharmacy and Odonto-Stomatology and the approval has been obtained. The IRB document includes details on the operational protocol and information on the pesticides to be used.

4. Results

4.1. Training

The training of trainers was conducted in 2008 and was offered to the MRTC members by Peter DeChant an expert in larviciding from the company ValentBiosciences based in Chicago, Illinois, United States of America (Mr DeChant's is the Business Development Manager of the Public Health and Forestry Business Unit at Valent BioSciences Corporation 16443 SE Meadowland Ct Portland, OR 97236 USA, he could be reached at 503-705-5401 Mobile, 503-618-8113 Office 925-817-5928 fax). Five staff members from MRTC were trained along with select participants (four per village to be having larviciding). They are Mamadou B. Coulibaly, Brehima Diallo, Amadou Guindo, Mohamed M. Traore and Amadou S. Traore. The sessions were conducted under the supervision of Pr. Seydou Doumbia, deputy director of the MRTC-entomology core.

The training program spanned over a day and included an interactive lecture and practices. The interactive lecture included:

- larviciding (breeding site identification, classification and treatment; risks and protection measures)
- description of the material used for larviciding and their maintenance.
- Description of the pesticides to be used (*Bacillus thuringensis* and *Bacillus sphaericus*) and the mechanisms of action.

The practical part of the training included:

- getting to know the sprayers (getting the names of the different parts)
- calibrating the sprayers
- doing the actual spray
- cleaning and storing the sprayers

The training of the spray operator took place on June 4th 2009 with the same training program but with some overviews on malaria and its control strategies. The program was as follows:

- overview on malaria (parasite, vector and transmission, and statistics)
- overview on malaria control strategies in general and in Mali
- larviciding (breeding site classification, identification and treatment; risks and protection measures)
- description of the material used for larviciding and their maintenance.
- Description of the pesticides to be used (*Bacillus thuringensis* and *Bacillus sphaericus*)

The practical part of the training included:

- getting to know the sprayers (getting the names of the different parts)
- calibrating the sprayers
- doing the actual spray
- cleaning and storing the sprayers

Ten (10) spray operators per village have been trained. Larviciding is to be implemented in three villages. Therefore thirty (30) spray operators have been trained among which two women. The women were interviewed to check if they were pregnant before they were enrolled. The training was done by the MRTC staff members (Dr. Coulibaly Mamadou, Dr. Diallo Brehima, Dr. Guindo Amadou, Dr. Traore Mohamed M., Mr Amadou S. Traore and Mr. Mamadou Konate) and the head of the health zone of Sirakorola (Dr. Michel Samake). Figure 3 and 4 show some moments of the training.

NB: 1. Even though larviciding was new to all of the select sprayers, many of them had very good experiences in spraying on crops; 2. the training was done in Bamanan, the local language (more 90% of Malians speak this language).

Fig 3: The training of the trainers (TOT). **A**: the Principal investigator (Mamadou Coulibaly standing left) introducing the MRTC-Entomology deputy director (seated right and facing) and the trainer (seated left and facing) before lectures start. **B**: The trainer is doing demonstrations. **C**. The trainees are practicing starting with the PI. **D**: the PI translating to the group the trainer's instructions in the local language.

Fig 4: Training of the spray operators: **A**: Dr. Michel Samake, head of the community health zone presenting the national malaria control strategies. **B**: Dr. Amadou Guindo from MRTC presenting the sprayers and their accessories and explaining how to use and clean them. **C & D**: Group preparing for practice. **E**: Group practicing (individually) spraying imaginary big water body or many small but connected or very close breeding sites. **F**: Group practicing on an imaginary breeding site that is big and deep at the center but shallow at the edges.

4.2. Identification, characterization and mapping of breeding sites

A map including all identified breeding sites for mosquito larvae, references to help localizing these sites and secondary road within the village has been produced for each village (see figures below). A plate with the respective number has been planted next to each identified breeding site.

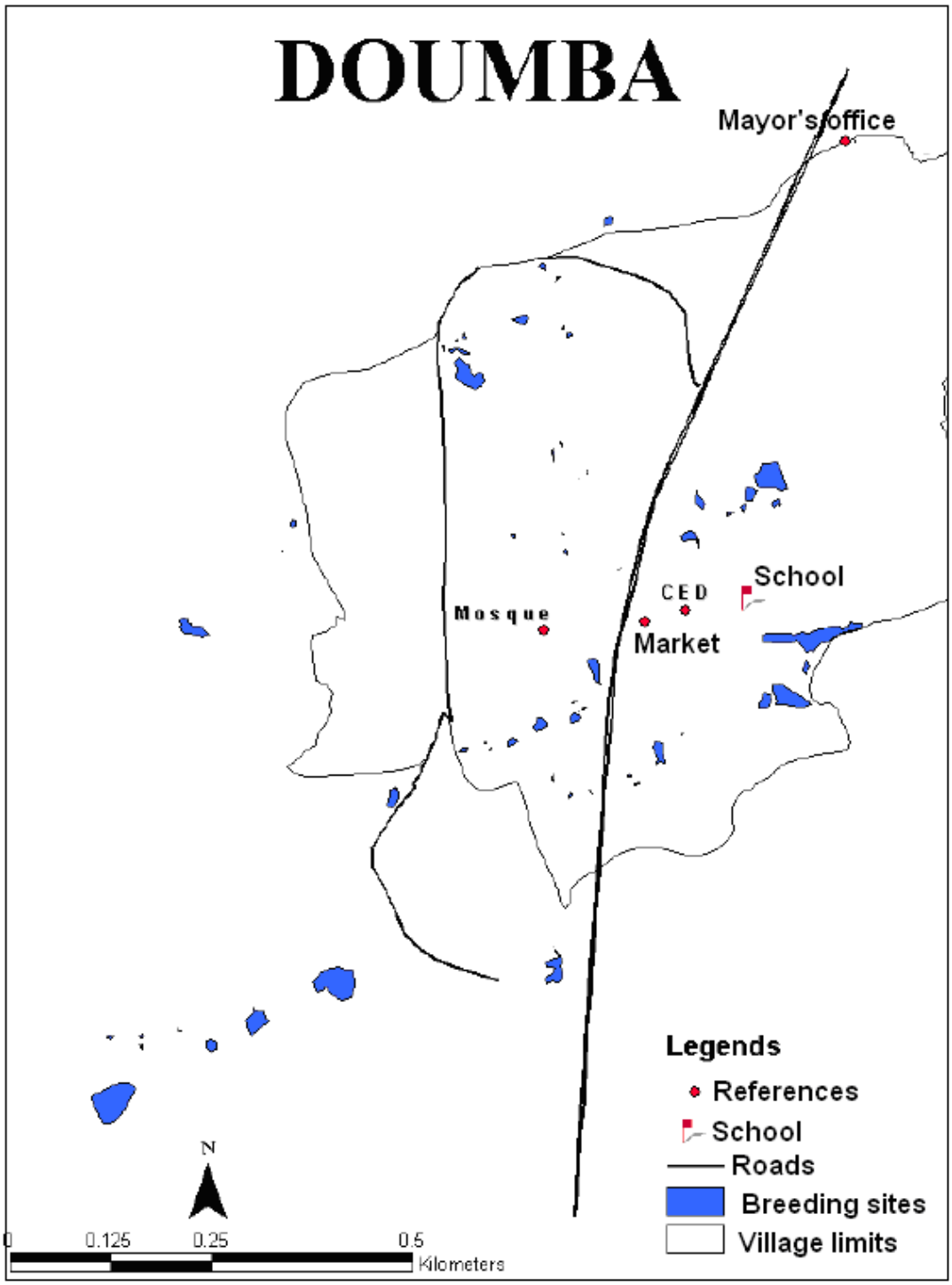


Figure 5: Map of identified breeding sites in the village of Doumba

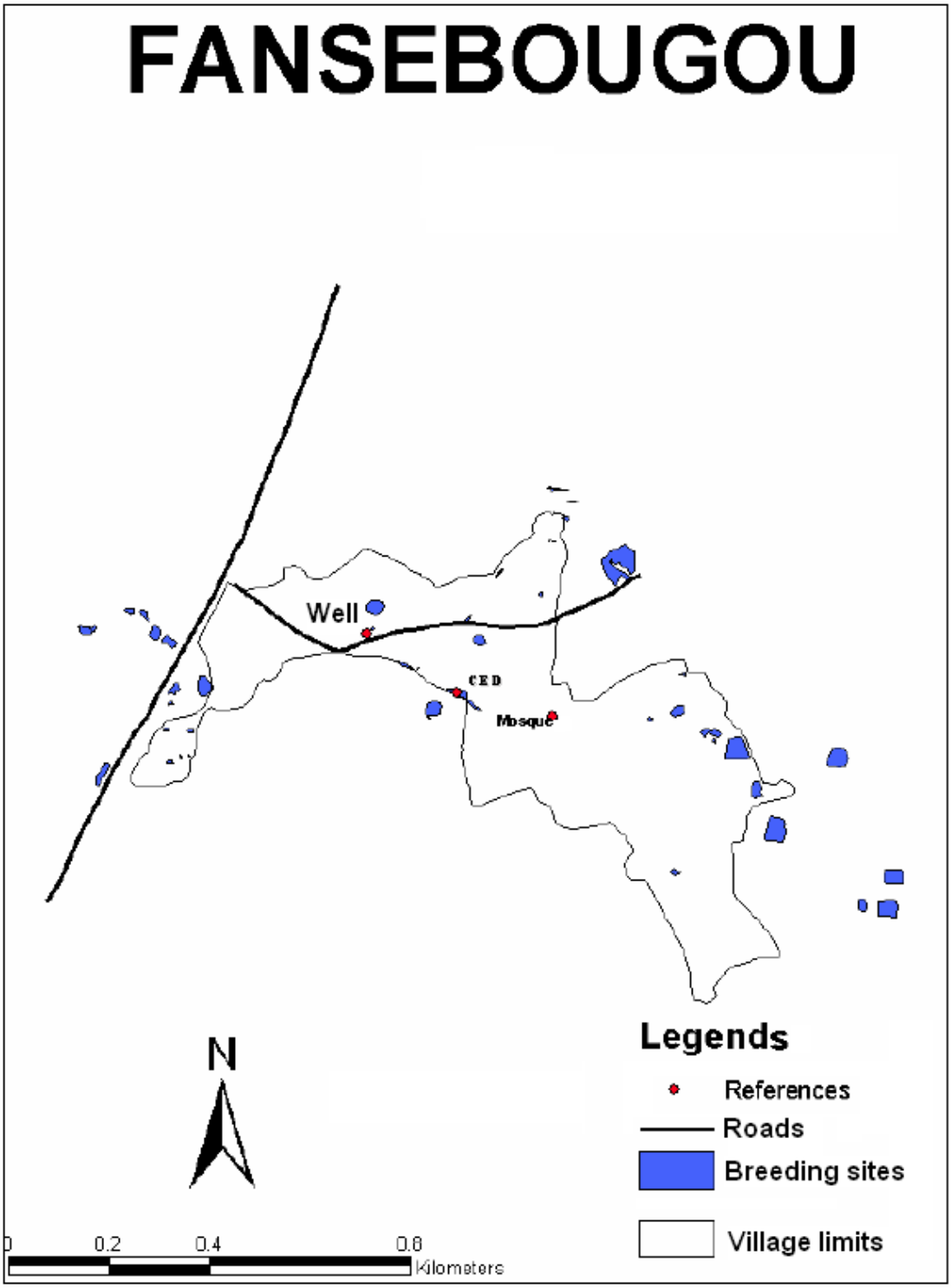


Figure 6: Map of identified breeding sites in the village of Fansebougou

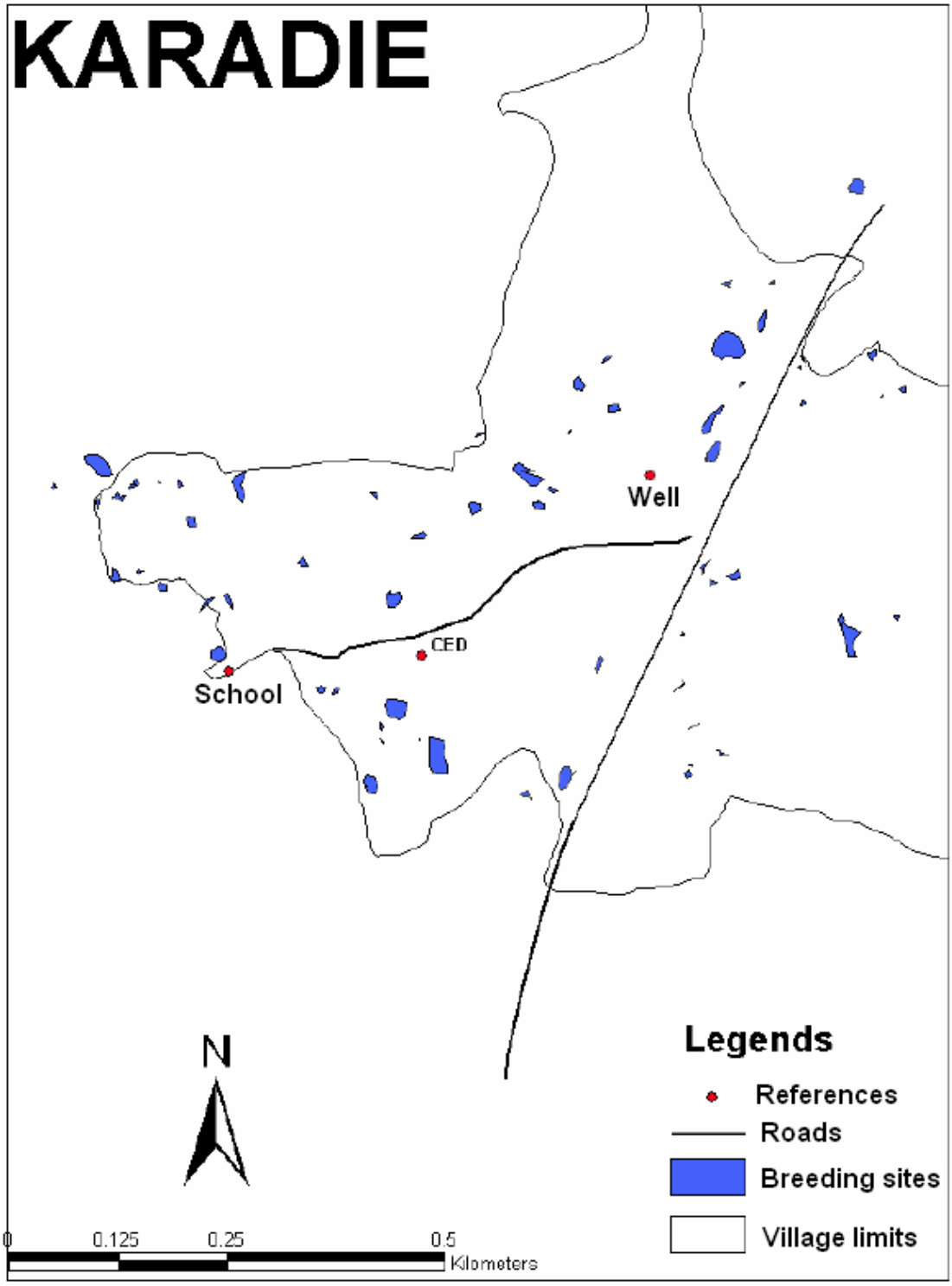


Figure 7: Map of identified breeding sites in the village of Karadie

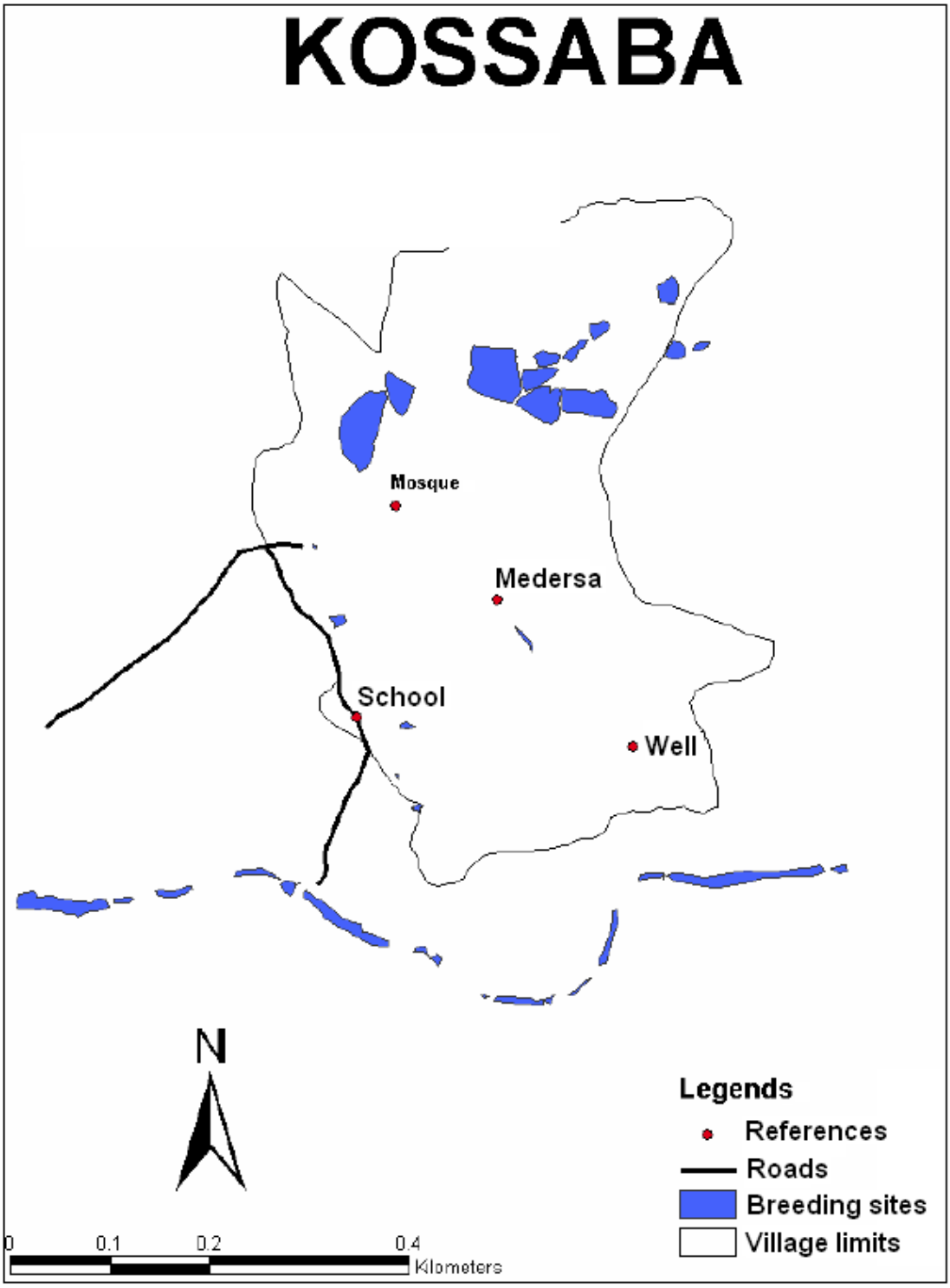


Figure 8: Map of identified breeding sites in the village of Kossaba

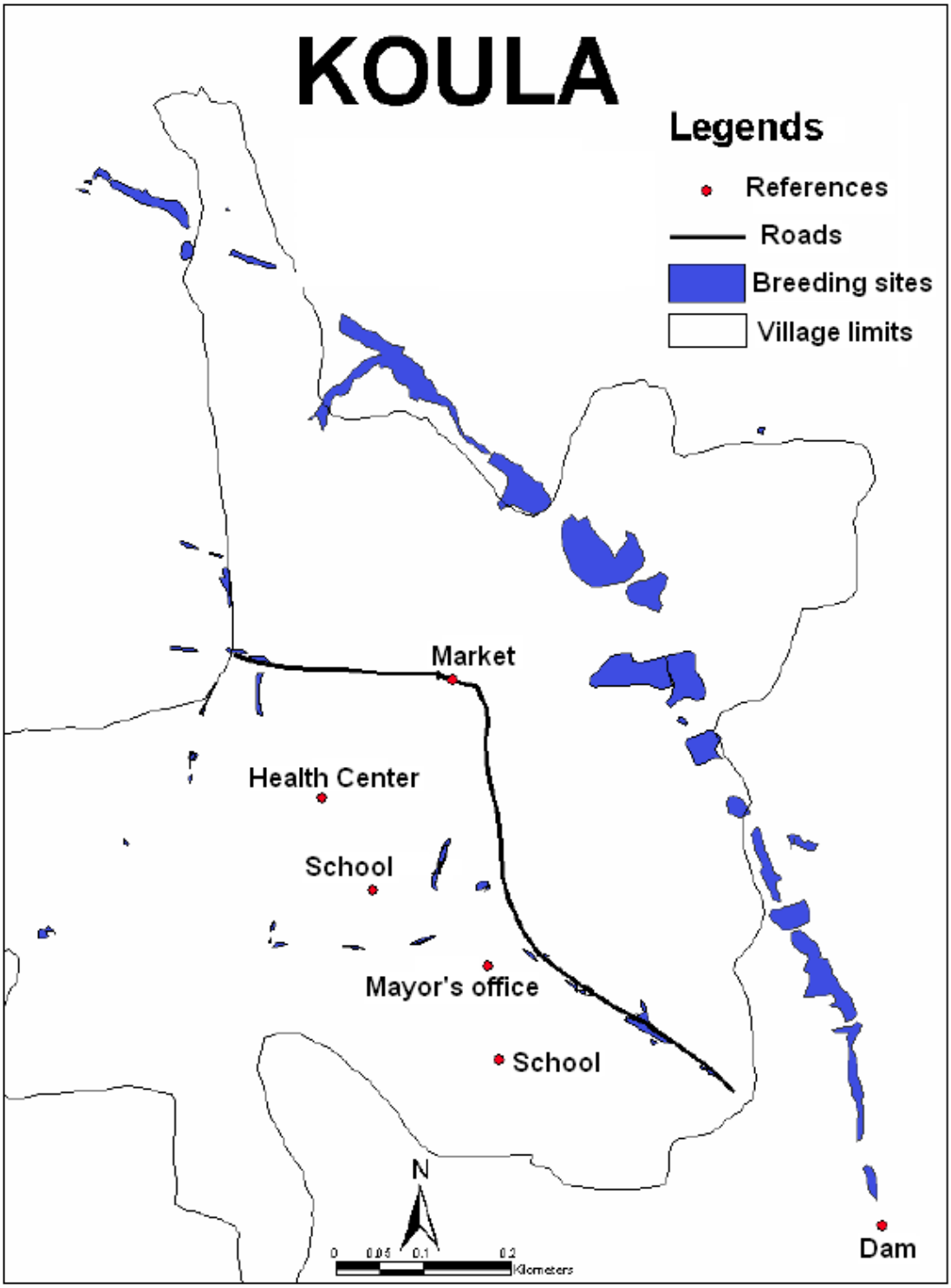


Figure 9: Map of identified breeding sites in the village of Koula

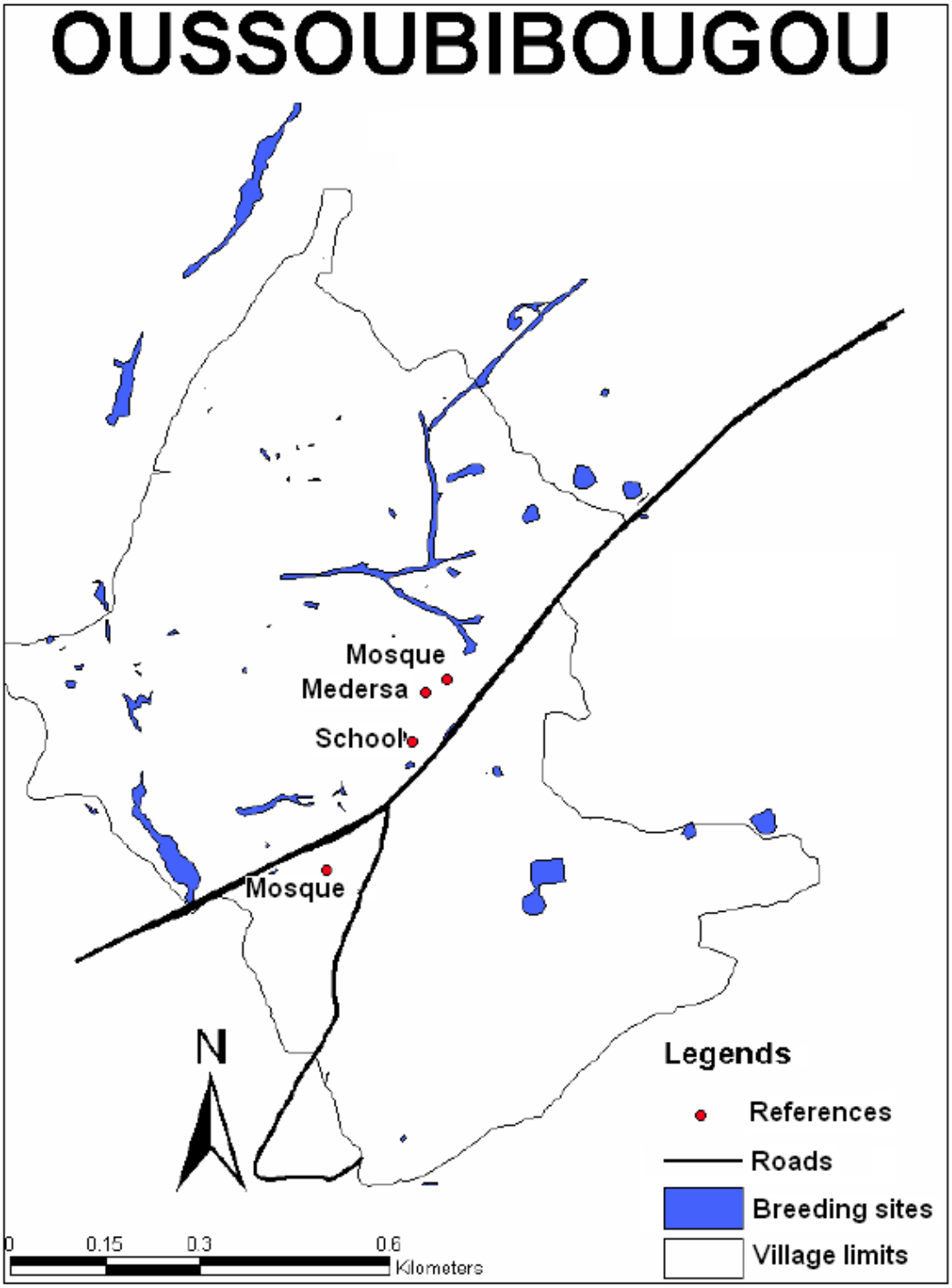


Figure 10: Map of identified breeding sites in the village of Oussoubibougou

These figures show outlines of the villages. At the end of breeding site identification a total of 140 were recorded (containing water or not) over the six villages. The table below provides the number of identified breeding sites per village containing water (42/140) at that moment. All of the latter contained mosquito larvae at different instars (from the first to the fourth).

Table 2: Frequency of breeding sites containing water per village.

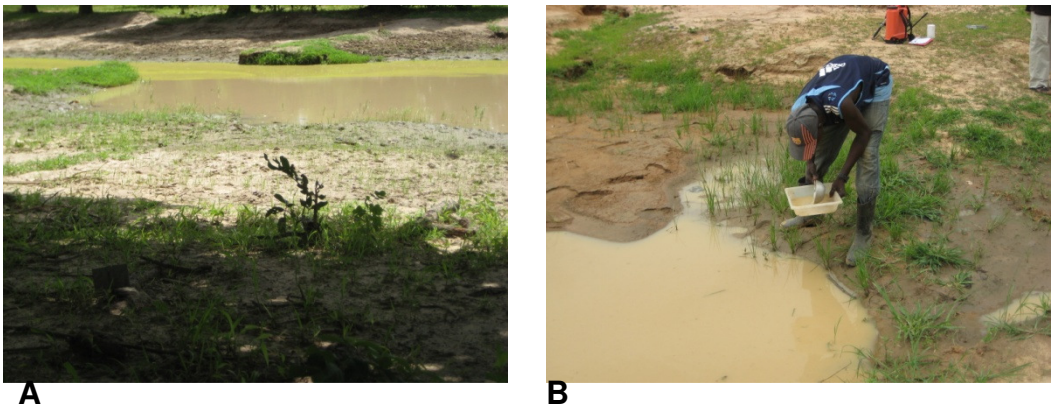
Villages	Nb breeding sites containing water (%)	Total
Koula	5 (14.3)	35
Doumba	4 (16)	25
Kossaba	6 (31.6)	19
Karadie	4 (21.1)	19
Fansebougou	8 (36.4)	22
Oussoubibougou	15 (75)	20
Total	**42 (30)**	**140**

Nb=number, %=percentage

Table 2 and Figure 12 show that man-made types of breeding sites predominate in the study sites. Figure 10 illustrates some recorded breeding sites.

Table 3 Types of breeding sites per village

Villages	Brick pits	Animal footprints	Tire prints	Streams	Natural depressions (ponds)	Total
Koula	3	0	19	1	12	35
Doumba	16	0	2	0	9	25
Kossaba	10	0	0	0	9	19
Karadie	19	0	0	0	0	19
Fansebougou	6	0	4	0	12	22
Oussoubibougou	18	1	1	0	0	20
Total	72	1	26	1	42	140



A B

Figure 11: **A**: Stream, receded because of drought leaving water pockets that became very productive breeding sites. Note the identification plate in the shade. **B**: Brick pit.

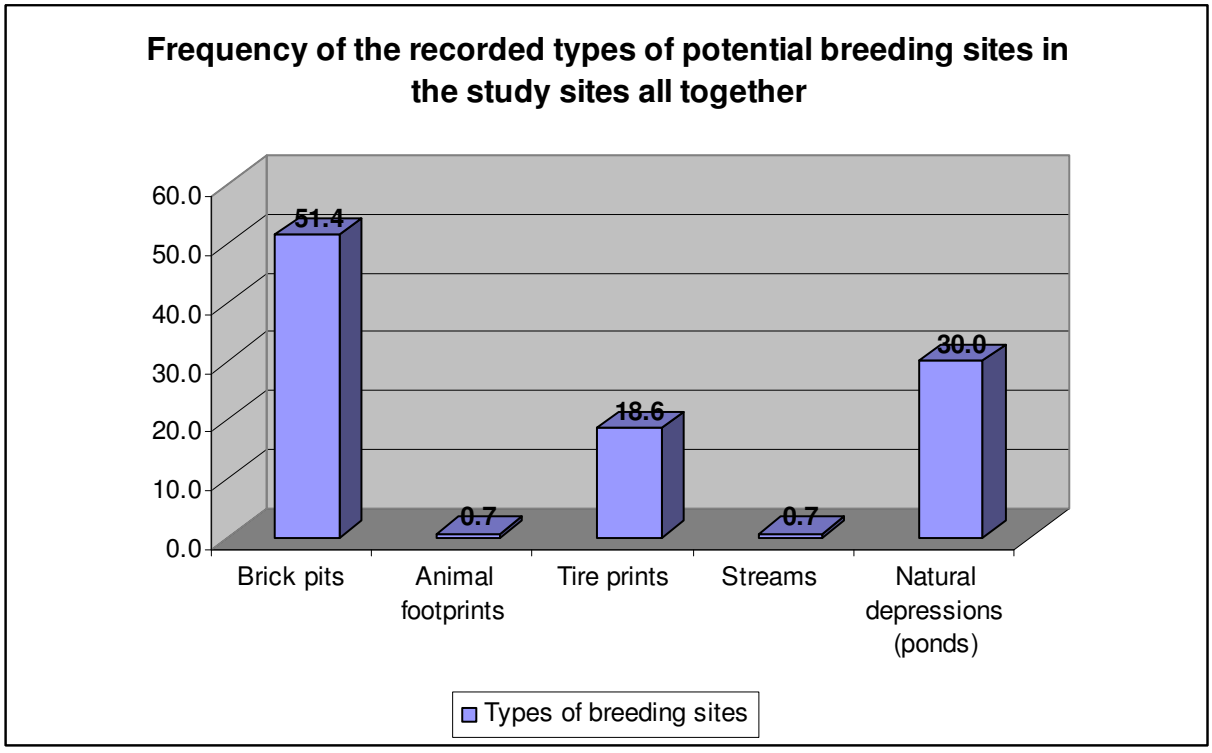


Figure 12: Frequency of the types of breeding sites

Brick pits represented 51.4% of all the potential breeding sites that were recorded. Natural depressions or ponds represented 30% while the tire prints represented 18.6%. Streams and animal footprints were rare to see.

4.3. Larviciding

4.3.1. Number of newly identified mosquito breeding sites

During the first week we recorded all identified potential breeding sites regardless the presence or absence of water within each select village (test and control) and 300m outside (from the last houses). In the subsequent weeks only wet potential breeding sites were recorded as new breeding sites. The figure below shows the number of new breeding sites recorded in the villages per month.

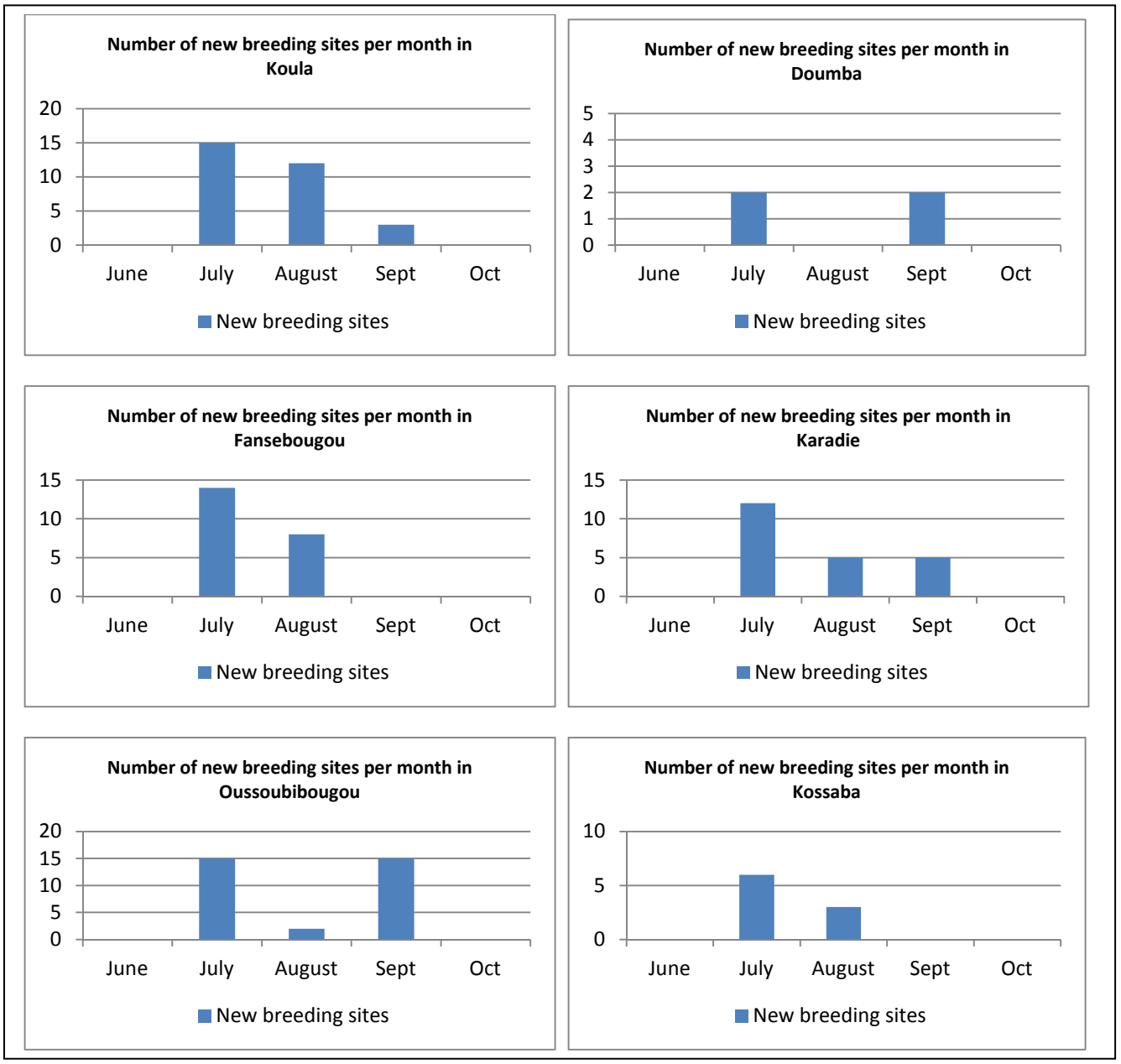


Fig 13: Number of new breeding sites per month and per village

Table 4 Number and frequency of wet breeding sites in all select villages per month

Villages		June	July	August	Sept	Oct
Koula	**Total**	35	43	55	58	58
	% wet	14.29	100.00	96.36	87.93	27.59
	New breeding sites	0	15	12	3	0
Fansebougou	**Total**	22	22	30	30	30
	% wet	36.36	100.00	76.67	100.00	40.00
	New breeding sites	0	14	8	0	0
Oussoubibougou	**Total**	20	38	40	55	55
	% wet	75.00	78.95	72.50	47.27	27.27
	New	0	15	2	15	0
Doumba	**Total**	25	27	27	29	29
	% wet	16.00	29.63	29.63	41.38	6.90
	New	0	2	0	2	0
Karadie	**Total**	19	34	39	44	44
	% wet	21.05	55.88	53.85	50.00	22.73
	New	0	12	5	5	0
Kossaba	**Total**	19	20	23	23	23
	% wet	31.58	50.00	100.00	78.26	4.35
	New	0	6	3	0	0

4.3.2. Reduction in larval presence in treated villages

The figure below shows the presence/absence of mosquito larvae before (monitoring) and after treatment (evaluation) with bio-pesticides in each of the three villages where larviciding was conducted. From June to October *Bacillus thuringiensis israelensis* (VectoBac®) was used at a weekly basis. In October, when there was less rain, *Bacillus sphaericus* (VectoLex®) was used fortnightly.

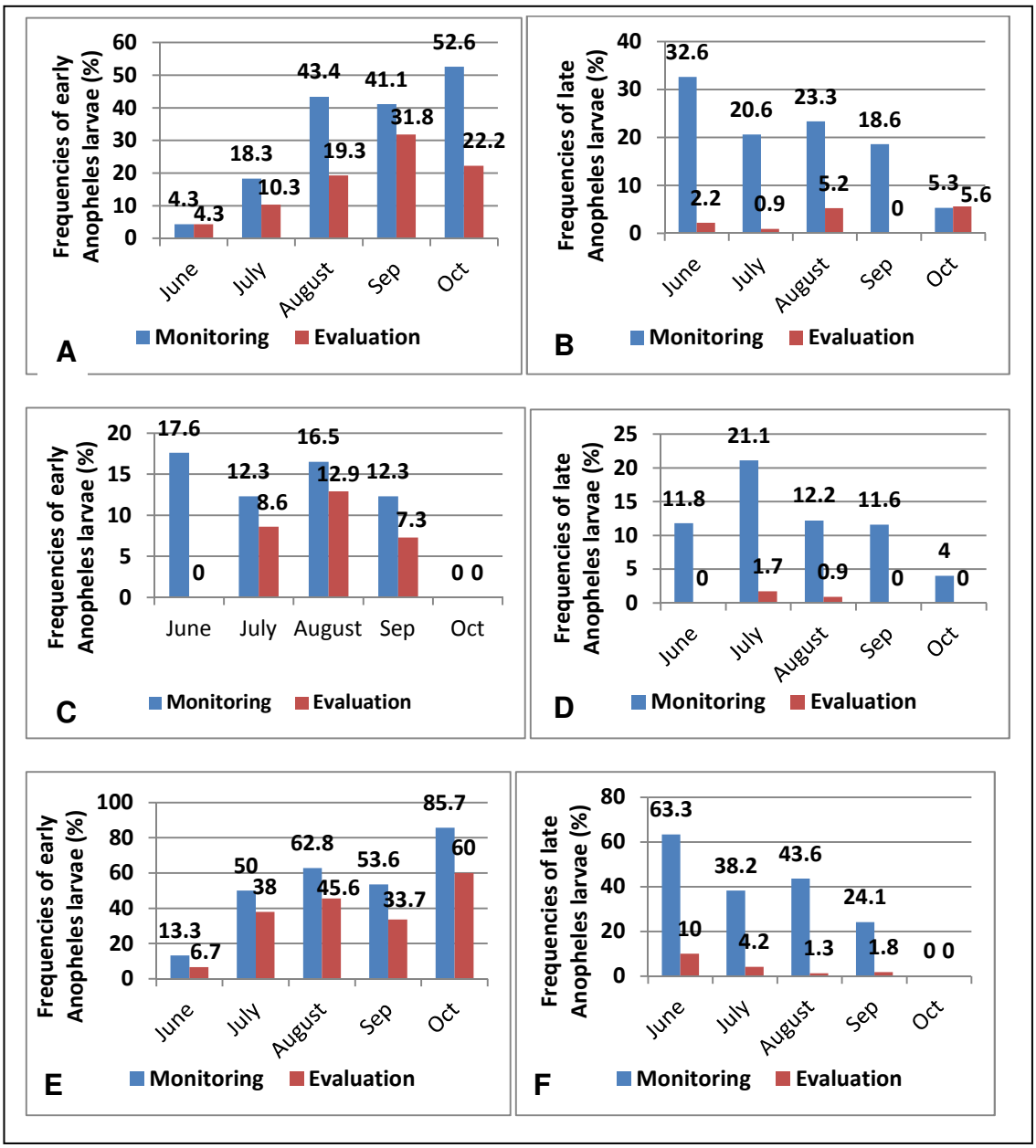


Fig 14: Frequency of early and late instar Anopheles before and after treatment in the test villages. Monitoring=before; Evaluation=after. **A**: early instars in Koula. **B**: late instars in Koula. **C**: early instars in Fansebougou. **D**: late instars in Fansebougou. **E**: early instars in Oussoubibougou. **F**: late instars in Oussoubibougou.

In all the three villages early instars of Anopheles (fig4) and Culicinae (fig 5) larvae were observed in breeding sites at both monitoring and evaluation from June to October. The frequencies were higher at monitoring than at the evaluation in each of the villages. However in the majority of the villages the difference was not significant. In contrast comparison of the frequencies of late instar Anopheles and Culicinae larvae showed significant reductions in all three villages with a few exceptions (September in Oussoubibougou and October in Koula).

NB1: Mosquito larval development requires three molts after the eggs hatched. That leads to four stages referred to as instars. The stage immediately after hatching is the first instar. In this document "Early instar" refers to the first and second instars; "Late instar refers to the third and fourth instar).

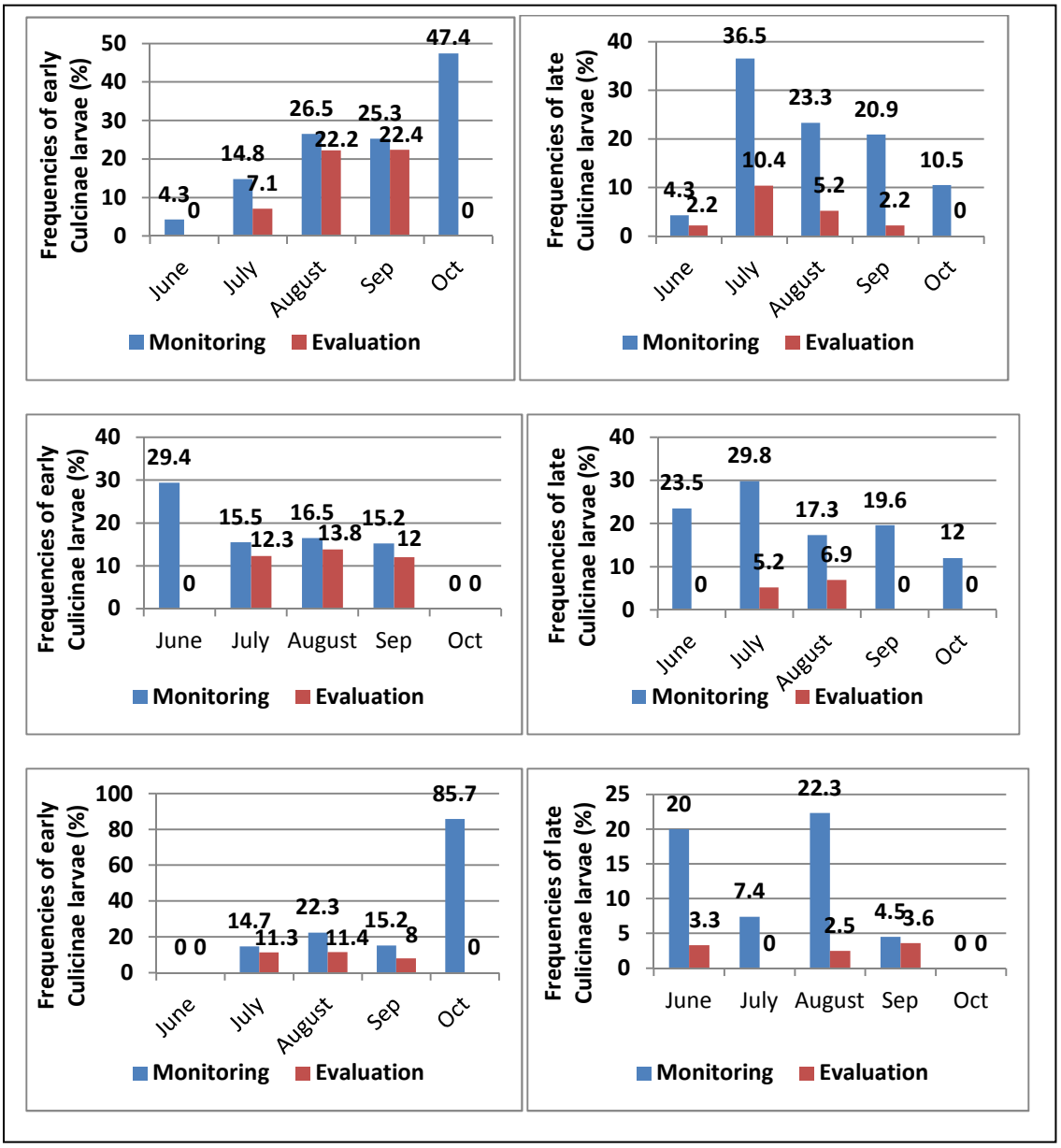


Fig 15: Frequency of early and late instar Culicinae before and after treatment in the test villages. Monitoring=before; Evaluation=after. **A**: early instars in Koula. **B**: late instars in Koula. **C**: early instars in Fansebougou. **D**: late instars in Fansebougou. **E**: early instars in Oussoubibougou. **F**: late instars in Oussoubibougou.

NB2: For the numbers and statistics see the attached supplements

4.3.3. Larval presence in test and control villages

Twelve breeding sites were randomly selected per village and visited per week to compare the presence/absence of early and late instar Anopheles and Culex between the test villages (IRS+Larviciding) and the control villages (IRS only). Early instars were encountered in almost all weeks and in all villages (see sections above). Densities of late instar Anopheles were compared in villages from July to October as in June and November we did not have enough productive breeding sites. Results showed a 100% reduction in late instar Anopheles larval densities.

Larval densities were measured per dipping using a 300ml dipper. In general a minmum of 10 dips was required per site but he number was adjusted when breeding sites were very big or very small. However the twelve sentinel sites were randomly selected among those estimated to have comparable sizes. So, ten dippings were done at each sentinel site. Tables 1-6 show the densities of late instar Anopheles larvae in the control villages (Doumba, Karadie and Kossaba) and in the test villages (Koula, Fansebougou and Oussoubibougou). In all the three test villages we observed a 100% reduction in the late instar larval densities from July to October.

Table 5-10 Densities of late instar *Anopheles* larvae in the control villages (Doumba, Karadie and Kossaba) and in the test villages (Koula, Fansebougou and Oussoubibougou) from July to October

Table 5

Doumba			
Months	Mean	N	SD
July	0.688	48	1.826
August	0.452	48	1.239
September	1.027	48	2.314
October	0.723	48	1.703

Table 6

Karadie			
Months	Mean	N	SD
July	0.471	48	0.676
August	1.727	48	2.889
September	1.606	48	2.783
October	0.565	48	0.981

Table 7

Kossaba			
Months	Mean	N	SD
July	1.271	48	1.272
August	3.881	48	3.834
September	5.45	48	3.218
October	2.356	48	4.011

Table 8

Koula			
Months	Mean	N	SD
July	0.123	48	0.289
August	0	48	0
September	0	48	0
October	0	48	0

Table 9

Fansebougou			
Months	Mean	N	SD
July	0.375	48	1.105
August	0	48	0
September	0	48	0
October	0	48	0

Table 10

Oussoubibougou			
Months	Mean	N	SD
July	0.589	48	0.899
August	0	48	0
September	0	48	0
October	0	48	0

NB 3: in the tables, N refers to the number of times the twelve breeding sites were monitored per month. For the test villages the evaluation data were not included. Therefore in a month there have been four visits per breeding sites hence the total number of 48. Also data from June and November were excluded as only a single trip was done and we did not have enough productive breeding sites to conduct any meaningful analysis.
SD refers to standard deviations.

4.3.4. Treatment

The actual larviciding was conducted in the three test villages. All the identified breeding sites were treated with *Bacillus thuringiensis israelensis* also known as *Bti* (VectoBac®). The treatment was conducted every week. Fig 10 shows operators treating breeding sites

Fig 16: Operators treating breeding sites with bio-pesticides.

4.3.5. Vector species identification and composition

Adult mosquitoes were collected by pyrethrinum spray catches (PSC) in twelve randomly selected houses in each of the six villages. The collection was organized fortnightly in the same houses. Table 7 shows the species composition of malaria vectors in the select villages from July to September. In all the six villages and during each month where mosquitoes were collected *An. gambiae s.s* represented the majority over *An. arabiensis*.
The *An. gambiae s.s.* specimens were object to further identification aiming at determining the molecular forms. Results showed that All *An. gambiae s.s.* assayed by PCR were of the M molecular form except two (2) in Karadie in September. These two were of the S molecular form.

4.3.6. Densities and man biting rate (MBR)

The adult *Anopheles gambiae* densities and MBRs are shown in Table 8. The MBRs were determined by dividing the number of fed and half-gravid mosquitoes (Table 9) by the number of people having slept in the rooms the previous night. These results show a considerable variation of these parameters across all the sites regardless the type of intervention (i.e. IRS versus IRS +Larviciding).

4.3.7. Infection rates and Entomological inoculation rates (EIR)

Table 10 shows the *Plasmodium falciparum* infection rates and the entomological inoculation rates in *Anopheles gambiae*. A considerable variation was observed in both parameters across all the sites regardless the type of intervention (i.e. IRS versus IRS + Larviciding).

Table 11 Vectorial composition per village from July to September in all six villages after PSC collections

Villages	July			August			September		
	***An. gambiae s.s.* (%)**	***An. arabiensis* (%)**	**Total**	***An. gambiae s.s.* (%)**	***An. arabiensis* (%)**	**Total**	***An. gambiae s.s.* (%)**	***An. arabiensis* (%)**	**Total**
Koula	10(100)	0	**10**	49(100)	0	**49**	44 (97.8)	1(2.2)	**45**
Fansebougou	13(92.9)	1(7.1)	**14**	0	0	**0**	0	0	**0**
Oussoubibougou	11(78.6)	3(21.4)	**14**	27(96.4)	1(3.6)	**28**	4(80.0)	1(20.0)	**5**
Doumba	9(90)	1(10)	**10**	23(100))	0	**23**	7(100)	0	**7**
Karadie	17(94.4)	1(5.6)	**18**	0	0	**0**	16(94.1)	1(5.9)	**17**
Kossaba	26(92.9)	2(7.1)	**28**	33(94.3)	2(5.7)	**35**	17(85.0)	3(15)	**20**
Total	**86(91.5)**	**8(8.5)**	**94**	**132 (97.8)**	**3(2.2)**	**135**	**88(93.6)**	**6(6.4)**	**94**

Table 12 Densities (D) and man biting rates (MBR) in the six villages per month, from July to September

Months	**July**				**August**				**September**			
Villages	**Nb sleepers**	**Total Collected**	**D**	**MBR**	**Nb sleepers**	**Total Collected**	**D**	**MBR**	**Nb sleepers**	**Total Collected**	**D**	**MBR**
Koula	98	19	0.79	0.15	98	132	5.50	1.06	98	23	0.96	0.20
Fansebougou	88	15	0.63	0.09	88	69	2.88	0.52	88	3	0.13	0.03
Oussoubibougou	93	23	0.96	0.14	93	34	1.42	0.29	93	4	0.17	0.04
Doumba	85	10	0.42	0.12	85	26	1.08	0.29	85	7	0.29	0.08
Karadie	71	33	1.38	0.34	71	221	9.21	2.28	71	20	0.83	0.20
Kossaba	86	36	1.50	0.36	86	112	4.67	1.01	86	26	1.08	0.24

Table 13 Number of fed and half-gravid female Anopheles gambiae collected by PSC per month and per village, from July to September

Months / Villages	July	August	September
	An. gambiae **Fed + half-gravid**	*An. gambiae* **Fed + half-gravid**	*An. gambiae* **Fed + half-gravid**
Koula	15	104	20
Fansebougou	8	46	3
Oussoubibougou	13	27	4
Doumba	10	25	7
Karadie	24	162	14
Kossaba	31	87	21

Table 14 Infection rates and entomological inoculation rates in the six villages per month from July to September

Months / Villages	**July**			**August**			**September**		
	Total	**Infection rate % (nb)**	**EIR**	**Total**	**Infection rate % (nb)**	**EIR**	**Total**	**Infection rate % (nb)**	**EIR**
Koula	18	5.56(1)	0.25	73	0	0	52	3.85(2)	0.23
Fansebougou	15	6.67(1)	0.18	42	0	0	22	0	0.00
Oussoubibougou	24	4.17(1)	17.51	33	6.06(2)	1.93	31	6.45(2)	0.08
Doumba	10	10.00(1)	0.36	26	0	0	15	0	0.00
Karadie	32	0	0.00	121	0.83(1)	0.57	72	4.17(3)	0.25
Kossaba	36	2.78(1)	0.30	59	0	0.00	75	1.33(1)	0.10

5. Information and sensitization on the importance of larviciding

This activity targeted two focus groups: the politico-administrative and opinion leaders at the region level, and the community leaders at the commune level. The goal was: i) to bring awareness on the role that communities inadvertently play in creating and maintaining mosquito breeding sites and ii) provide information on larval sources managements.

We expect to have a cascade communication (from the leaders to the populations) for a better understanding of the communities' role in mosquito breeding sites management as their participation will undoubtedly contribute to reducing mosquito densities.

Information and sensitization of the politico-administrative leaders

This was done through a meeting held in Koulikoro. The meeting was jointly organized by the Governor's office, the Regional Health Office, the NMCP and MRTC. On May 17th, 2010 a delegation of five and four persons from MRTC and NMCP respectively traveled to the district of Koulikoro to meet the other two groups cited above. The meeting started with the welcome speech of the mayor of the Koulikoro district followed by that of the representative of the director of NMCP. Then the governor's representative gave his speech and opened the sessions. Dr. Sidibé Halidou presented the national malaria control strategies and some recent results in malaria control. According to Dr. Sidibe the main control strategies are:

Intermittent preventive treatment in pregnant women (IPTp) using sulfadoxine-pyrimethamin (SP);
Rapid diagnostic;
Treatment with ACTs (uncomplicated malaria) and quinine (complicated malaria)
Large distribution of long lasting insecticidal nets (LLINs);
Indoors residual spraying;
Larviciding (operational research ongoing).

After Dr. Sidibe's presentation there was 30 minutes of questions and answers. Then Dr. Coulibaly presented on the malaria vectors in Mali and mosquito larval control. He gave a succinct description of the two major malaria vectors in Mali, *Anopheles gambiae s.l* and *An. funestus s.l*., their resting and biting behavior as well as their bionomics. Accent was made on the breeding sites. Participants were shown differences between the preferred breeding sites for *Anopheles* and the preferred ones for *Culex* and *Aedes*. He stressed out that most of breeding sites were created by human activities (e.g. brick pits, tire prints etc.).

Dr. Coulibaly described the different malaria control methods with an emphasis on larval sources management. Participants were informed about the different larval sources control methods. These include:

Physical destruction of breeding sites
Larviciding (chemical and biological products)
Use of organisms such as fishes and fungi to control larvae
Environment management.

A question/answer (Q&A) session followed for 45-60 minutes. The meeting was then suspended for a break. After the break Dr. Coulibaly presented some results of the pilot study on larviciding. These results are those given in section 4.3.. Another Q&A session

followed. After that session a list of recommendations was made by the participants and the speakers. Below is the list.

Table 15 Closing recommendations and the indicated responsible

Recommendations	Responsible
Relay information on larval sources management to the communities	Regional Health Office (Director)
Take over vector control strategies including larviciding in the Koulikoro district	Mayor's office
Create a malaria control coordination organ in Koulikoro	Governor's office (governor)
Provide resources for communication on malaria mostly for associations and community agents, etc.)	Regional office for social development (Director)
Promotion of larval sources management	Regional Health office (Director)
Lobby and mobilize financial resources for larval sources management	The Governor's office (The governor) Regional Parliament (the president) Regional Health Office (the Director)
Finalize the pilot study on larval sources management	MRTC
Develop partnership with territorial collectivities and technical services for environment management and malaria control	The governor's office (the governor) Regional environment management office (the director)

Information and sensitization of the community leaders from the study sites

This meeting was held on Thursday June 03rd, 2010 in Sirakorola, one of the two communes where larviciding is being conducted. The following leaders were invited for each of the six villages participating in the Larviciding study: the chief of the village, the leader of the youth, the women's leader and the principal guides helping with the study. As political figures the mayors and the sub-prefects were invited. The policy of the meeting was held by a representative of the district health center and the head of community health center of Sirakorola.

Dr. Sidibé Halidou and Dr. Coulibaly were the speakers and the language was Bambara. The community leaders were informed on the role of communities in creating mosquito breeding sites. They were also informed on different methods of larval sources management. The same materials as for the politico-administrative leaders were used with the difference in the language.

The main recommendation was for every participant to be an ambassador for larval sources management not only in her/his village but also wherever possible.

6. Conclusions

Four of the MRTC staff members were trained on how to conduct larviciding. They received lectures on the pesticides (*Bacillus thuringiensis* and *Bacillus sphaericus*) to be used for the project as well as their mechanisms of action. They were trained on how to calibrate the sprayers.

Thirty spray operators, ten per village, have been trained on how to conduct larviciding. The group includes two women. Though this gender rate looks biased it is a very good start in a place where women would not be called on for such activities. One of the advantages of this training is that trainees come from the villages. Therefore the acquired competencies stay in the villages. That will be an added value to the education of the villages in terms of malaria control strategies.

Regarding mapping 140 potential breeding sites have been identified and mapped. Geographical coordinates have been recorded. However this number is subject to change as the rainy season starts and goes. This study shows that man-made breeding sites predominate in the study sites. However a great part of the tire prints (19) has been recorded in a single village (Koula). A possible explanation for that is that more cars might be coming to Koula than the other villages. That is because it is the principal village of a community of several villages and has the political offices and the health center while the other villages included in the study don't have that privilege. Therefore, though tire prints are considered good breeding sites, their high frequency (18.6%) is not random.

Overall the number of breeding sites increased from July to September though in some of our villages we did not record many or any in August. The number increases with the frequency and the abundance of the rain as the rainy season starts in June (very timid) peaks in August September and stops around October-November. Part of the reduction or the absence of breeding sites number could be explained by the fact that some of them are eliminated by plowing as people grow crops around houses.
It is noteworthy that we recorded fewer breeding sites than we expected. That could be an advantage for larviciding operations.

The larviciding operations did not have a huge impact on the frequencies of early instar larvae although we have seen a significant reduction in Oussoubibougou during August and September. However the impact was highly significant on the frequencies of late instar larvae regardless the species (Anophelinae or Culicinae). The before-and-after assessment of the frequencies of late instar larvae shows that larviciding reduces mosquito larval frequencies in test villages even when all identified productive breeding sites were considered.

Regarding the densities the study focused on the densities of late instar *Anopheles* in twelve sentinel productive breeding sites per village (this includes all six villages). Results show a 100% reduction in the densities after three treatments in tests villages whereas we continued to count late instar larvae in the sentinel sites located in the control villages.

The entomological parameters on adult mosquitoes (densities, MBRs, infection rates and the EIR) have shown considerable variations across all the villages and through time. Therefore a conclusion could not be drawn from comparison between test villages and control villages. Fluctuations might exist even in absence of any control measures. In addition the results could be influenced by adult mosquitoes migrating in the study areas or by individuals emerging from breeding sites that we could not identify.
The lack of baseline data makes it more complicated to infer a conclusion on the impact of this study on the adult densities, MBRs, infection rates and EIRs. Another difficulty is that the IRS operations were not uniform across houses and villages.

In the end though we could not draw a conclusion on the absolute added value of larviciding to IRS, with a 100% reduction of late instar larvae, at the sentinel sites, in both malaria vectors and nuisance mosquitoes we remain confident that larviciding has a relative added value to IRS. Therefore larviciding is promising and could be proposed to be integrated into the NMCP's strategic plan in regions where the majority if not all the breeding sites could be identified.

All this effort will not go far without the communities' participation. The series of two meetings (one for the politico-administrative and opinion leaders and one for the study sites communities) planned by MRTC/NMCP aim at informing and sensitizing the communities about the importance of larval sources management in controlling malaria. The first meeting intended for the leaders was very encouraging as most of the invited leaders were present and the sessions culminated with a list of recommendations to the regional administration and parliament as well as to the community leaders. The second meeting set for the villages, also, held its promises. Community leaders were informed and each of them is going to be an ambassador for larval source management. This is a sign that the Kouilkoro community is getting ready to take a big step in malaria control by engaging both authorities and communities. These actions need to be replicated at the national level.

7. Acknowledgments

- **Villages' members**
- **DPNLP**
- **RTI International Inc.**
- **USAID**
- **LMVR/NIAID/NIH**
- **CDC-Atlanta**
- **MRTC**
- **MSC**

- Mohamed M. Traoré, MD
- Mamadou Konaté, M.Sc
- Sekou A. Traoré, Student
- Bilkissou Yagouré, M.Sc
- Mahamoudou B. Toure, MD
- Daniel Szumlas, PhD, CDC
- Dansine Diarra (GIS)
- Nafomon Sogoba

Supplements

The supplements provide details on the frequencies of larvae in all identified breeding sites. N refers to the number of times breeding sites have been visited. “m” and “e” refer to monitoring and evaluation respectively. The numbers in the “m” and “e” columns represent the percentages of times an instar (early or late) larva is encountered. “P” is the probability showing the degree of significance (threshold=0.05) from the comparison of the larval frequencies before (monitoring) and after (evaluation) treating the breeding sites with larvicides.

S1. Frequencies of Anophelinae larvae in Koula, Fansebougou and Oussoubibougou

Koula										
Instars / Month	Early instars				P	Late instars				P
	m(%)	N	e(%)	N		m(%)	N	e(%)	N	
June	4.3	46	4.3	46	1.000	32.6	46	2.2	46	0.000
July	18.3	126	10.3	115	0.096	20.6	126	0.9	115	0.000
August	43.4	189	19.3	135	0.000	23.3	189	5.2	135	0.000
September	41.1	253	31.8	223	0.045	18.6	253	0.0	223	0.000
October	52.6	19	22.2	18	0.091	5.3	19	5.6	18	1.000

Fansebougou										
Instars / Month	Early instars				P	Late instars				P
	m(%)	N	e(%)	N		m(%)	N	e(%)	N	
June	17.6	17	0.0	17	0.227	11.8	17	0.0	17	0.485
July	12.3	58	8.6	57	0.369	21.1	58	1.7	57	0.001
August	16.5	139	12.9	116	0.482	12.2	139	0.9	116	0.000
September	12.3	150	7.3	138	0.168	11.6	150	0.0	138	0.000
October	0.0	25	0.0	25	-	4.0	25	0.0	25	0.500

Oussoubibougou										
Instars / Month	Early instars				P	Late instars				P
	m(%)	N	e(%)	N		m(%)	N	e(%)	N	
June	13.3	30	6.7	30	0.671	63.3	30	10.0	30	0.000
July	50.0	68	38	71	0.174	38.2	68	4.2	71	0.000
August	62.8	94	45.6	79	0.032	43.6	94	1.3	79	0.000
September	53.6	199	33.7	112	0.001	24.1	199	1.8	112	0.000
October	85.7	14	60.0	15	0.215	0.0	14	0.0	15	-

S1. Frequencies of Culicinae larvae in Koula, Fansebougou and Oussoubibougou

Koula										
Instars / Month	Early instars				P	Late instars				P
	m(%)	N	e(%)	N		m(%)	N	e(%)	N	
June	4.3	46	0.0	46	0.495	4.3	46	2.2	46	1.000
July	14.8	126	7.1	115	0.064	36.5	126	10.4	115	0.000
August	26.5	189	22.2	135	0.434	23.3	189	5.2	135	0.000
September	25.3	253	22.4	223	0.519	20.9	253	2.2	223	0.000
October	47.4	19	0.0	18	0.001	10.5	19	0.0	18	0.486

Fansebougou										
Instars / Month	Early instars				P	Late instars				P
	m(%)	N	e(%)	N		m(%)	N	e(%)	N	
June	29.4	17	0.0	17	0.044	23.5	17	0.0	17	0.051
July	15.5	58	12.3	57	0.409	29.8	58	5.2	57	0.000
August	16.5	139	13.8	116	0.602	17.3	139	6.9	116	0.010
September	15.2	150	12.0	138	0.492	19.6	150	0.0	138	0.000
October	0.0	25	0.0	25	-	12.0	25	0.0	25	0.117

Oussoubibougou										
Instars / Month	Early instars				P	Late instars				P
	m(%)	N	e(%)	N		M(%)	N	e(%)	N	
June	0.0	30	0.0	30	-	20.0	30	3.3	30	0.103
July	14.7	68	11.3	71	0.618	7.4	68	0.0	71	0.026
August	22.3	94	11.4	79	0.070	22.3	94	2.5	79	0.000
September	15.2	199	8.0	112	0.057	4.5	199	3.6	112	0.776
October	85.7	14	0.0	15	0.000	0.0	14	0.0	15	-